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(54) **Composition for treatment of ischemic disorder in organs.**

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Description

The present invention relates to the use of a composition for the preparation of a medicament for the prophylaxis and treatment of ischemic disorder in mammalian organs such as the heart.

Disease in mammalian organ such as the heart, brain, kidney, are often observed in adults.
This disease is mainly attributed to injury of cells or tissues, which are often caused by ischemia.
This injury is caused by lacking of energy supply, which is attributed to decrease or cease of blood flow.
The aggravation of ischemic tissue, i.e., a decline of cell function, injury of cell wall and destruction or necrosis of cell, depends upon the duration of the ischemia and the sensitivity to the ischemia of organ cells.

Therefore, vasodilators, β -blockers and calcium-antagonists has been used for prophylaxis and treatment of ischemic disorders in mammalian organs up to the present. Vasodilators increase the supply of blood to the organs. β -blockers and calcium-antagonists reduce the energy demand of organ cells and increase the tolerance of organ cells.

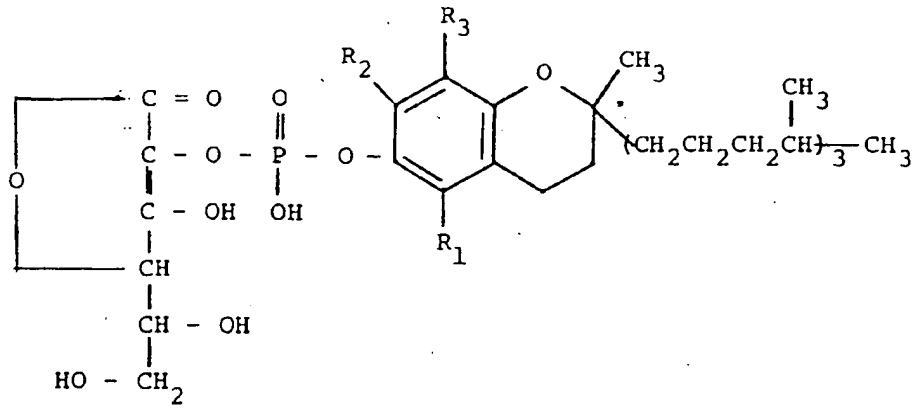
Chemical Abstracts, vol. 104 (1986), abstract no. 4935x), discloses that the physical mixture of the vitamin C and α-tocopherol inhibits lipid peroxydation more than α-tocopherol alone in patients with myocardial infarction (ischemic disorder). However, there is nothing to suggest the use of the compound or its salt, as used in the present invention.

J. Soc. Cosmet. Chem., vol. 38, no. 5 (1987), pages 333-339) employs a new provitamin (DL-α-tocopherol-L-ascorbic acid sodium phosphate diester). This reference discloses that the provitamin is stable in aqueous solutions and effectively bioconverted into both vitamins in the viable skin of hairless mice. Moreover, in the reference, the skin permeation and bioconversion of the provitamin is described using an in vitro skin permeation apparatus.

However, it has been revealed by the recent research that the formation of lipid peroxide from unsaturated fatty acid, which is a component of the cell wall, and the harmful action of physiologically active metabolites such as prostaglandins, leucotriens, are deeply concerned with injury of ischemic cells or tissues.
Though enormous effort has been devoted to find out and develop new types of drugs based on these recent basic researches, no drug has been found satisfactorily.

The present inventors have investigated the pharmacological action of phosphoric diester compounds wherein, among the three hydroxyl groups in the phosphoric acid, one hydroxyl group is esterified with the hydroxyl group on the 2-position of ascorbic acid and another hydroxyl group is esterified with the hydroxyl group in one of the tocopherols or their analogs, including α-tocopherol. It has been found that these compounds have other specific pharmacological activities, namely the activity to inhibit lipid peroxide formation and a reducing activity of the size of infarction in ischemia-reperfused rat heart. The present invention was accomplished based on the studies thus far made.

Thus, the present invention provides the use of a composition for the preparation of a medicament for the prophylaxis and treatment of ischemic disorders in mammalian organs which contains, as an ingredient, a compound of the formula



wherein R_1, R_2 and R_3 are independently the methyl group or a hydrogen atom, or a salt thereof.

Among the compounds (I), the compounds wherein at least one of R_1 and R_2 is the methyl group and R_3 is the methyl group are preferable.

The compounds (I) used in the present invention are known compounds as disclosed in the literature and are produced by, for example, reacting α-tocopherol with a halogenophosphorylating reagent such as phosphorous oxytrichloride, reacting the resulting product with ascorbic acid wherein the hydroxyl groups in the 5- and 6-positions are protected with a protecting group such as isopropylidene, and removing the protecting groups in the resulting product. The method of producing the compounds (I) is described in detail in U. S. Patent No. 4,564,686 and EP-A2-0236120.

Alkali metal salts such as the sodium salt and the potassium salt and alkaline earth metal salts such as the calcium salt and the magnesium salt may be exemplified as salts of the compounds (I).

As for the above compounds (I) and the salts thereof, L-ascorbic acid, DL-α-tocopheryl phosphate diester, its potassium salt, its sodium salt or its calcium salt; L-ascorbic acid, DL-β-tocopheryl phosphate diester or its sodium salt; L-ascorbic acid, D-γ-tocopheryl- phosphate diester or its sodium salt; L-ascorbic acid, D-δ-tocopheryl phosphate diester or its potassium salt; and L-ascorbic acid, DL-tocoryl phosphate diester or its sodium salt may be exemplified.

These compounds have been known as antiinflammatory agents, agents for the prophylaxis and treatment of cataracts and climacteric syndrome, and as ingredients for cosmetics such as those having a skin-beautifying action.

The composition for the prophylaxis and treatment of ischemic disorders in mammalian organs of the present invention contains the above-mentioned compound or salt thereof as an ingredient.

The present composition can be administered orally or parenterally to a mammal such as human beings. The composition may be administered in a form of an injectable solution, a tablet, a capsule or as a syrup by mixing with per se conventional pharmacologically acceptable carriers, i.e., excipients, diluents.

While the dosage varies with the subject, administration routes, symptoms, it is usually administered to an adult human in an amount of more than 0.1 mg/kg body weight one time, usually 5 to 1,000 mg/person, preferably 5 to 300 mg/person 1 to 3 times a day.

The compounds (I) or salts thereof which is the ingredient of the present composition demonstrate the activity to inhibit lipid peroxide formation in vitro by use of brain tissue homogenate and the activity to improve the cardiac disorder in ischemia-reperfusion rat model.

Furthermore, the toxicity of the ingredient of the present composition is extremely low, namely, for example, LD_{50} of L-ascorbic acid, DL-α-tocopheryl phosphate diester sodium salt is more than 10 g/kg (rat) by oral administration and 737 mg/kg (rat) by subcutaneous administration.

Therefore, the present composition is useful for prophylaxis and treatment of ischemic heart disease (myocardial infarction, heart failure, arrhythmia), ischemic disorders of cerebral tissue (cerebral infarction, cerebral apoplexy) and ischemic renal disorders (renal incompleteness) in mammals such as human beings.

EXAMPLES

The examples are given below to illustrate the present invention more specifically.

In the examples, the compound names are partly abbreviated in the following manner:

α-EPC-Na(DL) : L-Ascorbic acid, DL- -tocopheryl phosphate diester sodium salt [R_1, R_2, R_3 = CH_3]

β-EPC-K(DL) : L-Ascorbic acid, DL-β-tocopheryl phosphate diester potassium salt [R_1, R_3 = CH_3, R_2 = H]

γ-EPC-K(D) : L-Ascorbic acid, DL-γ-tocopheryl phosphate diester potassium salt [R_1 = H; R_2, R_3 = CH_3]

TPC-Na(DL) : L-Ascorbic acid, DL-tocoryl phosphate diester sodium salt [R_1, R_2, R_3 = H]

Example 1

Activity of the present compounds to inhibit lipid peroxide formation in rat brain tissue homogenate:

(i) Method:

Male SD rats (10 to 12 weeks old) were subjected to examination under anesthesia with pentobarbital, then the brain tissue was excised. The brain tissue was homogenized in a phosphate buffer solution (pH 7.4) to prepare a 5% homogenate. After incubation of the homogenate at 37 °C for 1 hour, the amount of malonic dialdehyde was determined by thiobarbituric acid (TBA) method in accordance with the report of Ohkawa et al., in Analytical Biochemistry, 95, 351 (1979). The amount was used as the index of the amount

of lipid peroxides formed therein.

The test drug was added to the 5% homogenate before incubation so as to make the final concentration of 10^{-4}M. The activity to inhibit the formation of lipid peroxide was compared with that of the reference group to which was added the solvent (DMSO), and shown by % inhibition.

(ii) Results:

The results are shown in Table 1 below.

Table 1

Inhibitory Effects of the Test Compounds on Lipid Peroxidation in Rat Brain Homogenate		
Compound	Inhibitory Effect (%)	Number of Experiments (n)
α-EPC-Na(DL)	61.8 ± 38.1	(4)
β-EPC-K(DL)	58.9 ± 19.4	(3)
γ-EPC-K-(D)	62.0 ± 8.2	(3)
δ-EPC-K(D)	38.1 ± 8.3	(3)
TPC-Na(DL)	35.7 ± 2.6	(3)
DL-α-tocopherol	50.8 ± 18.5	(4)
L-ascorbic acid	1.6 ± 15.0	(7)

As shown in the Table 1, the present hydrophylic compounds inhibited lipid peroxidation more than that of α-tocopherol which is lipophylic, and this indicates that the present compound is a useful agent. But L-ascorbic acid per se did not inhibit lipid peroxidation.

Example 2

Reducing activity of α-EPC-Na(DL) against the size of infarction in ischemic-reperfused rat heart:

Wistar male rats (body weight 276 - 3390 g) were anesthetized with pentobarbital and subjected to thoractonomy. The left anterior descending coronary artery (LAD) was ligated at its origin for 1 hour. Then, the ligation was released to allow reperfusion. After 30 to 60 minutes reperfusion, the chest was closed and the animals (rats) maintained in conscious state. After 24 hours, the animals (rats) were reanesthetized, and then, the heart was excised. The left ventricles were cut parallel to the atrioventricular sulcus into slices. The slices were stained at 37°C for 15 minutes using triphenyltetrazolium chloride, and the size of infarction was weighed.

α-EPC-Na dissolved in saline solution was administered into the femoral vein at doses of 0.3, 1 and 5 mg/kg 30 minutes after the ligation. For the control group, only saline solution was administered.

ii) Results:

The results were shown in Table 2 below:

Table 2

Reducing Activity of α-EPC-Na(DL) Against the Size of Infarction in Ischemic-Reperfused Rat Heart

Dose Amount (mg/kg,i.v.)	Number of Experiments (n)	Size of Infarction (% of left ventrical)	Inhibition (% of Control)
Control	(8)	35.0 ± 2.9*	
α-EPC-Na(DL)			
0.3	(4)	32.4 ± 4.3	- 7.4
1.0	(5)	27.2 ± 2.8	- 22.3
5.0	(5)	18.3 ± 1.1	- 47.7

* The figures are shown as mean value ± SEM.

As shown in Table 2, it was clarified that α-EPC-Na-(DL) reduced the size of myocardial infarction dose-dependently and to about 50% at a dose of 5 mg/kg.

Example 3

Tablets are prepared by the conventional method using the following components:

. L-ascorbic acid, DL-α-tocopheryl phosphate acid diester sodium salt	50 mg
. Corn starch	90 mg
. Lactose	30 mg
. Hydroxypropylcellulose	25 mg
. Magnesium stearate	5 mg
	Total 200 mg (a tablet)

Example 4

An injectable solution is prepared by the conventional method using the following components:

. L-ascorbic acid, D-δ-tocopheryl phosphate diester sodium salt	20 mg
. Glucose	5 mg
. Distilled water for injection	Total 100 ml

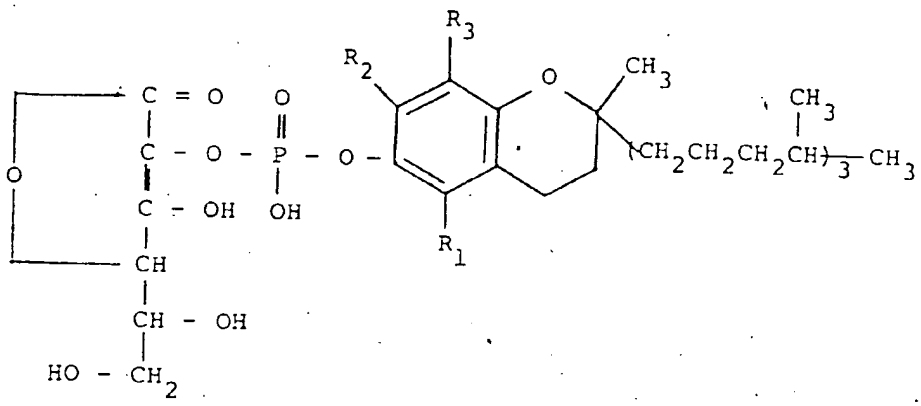
Example 5

Tablets are prepared by the conventional method using the following components:

. L-ascorbic acid, DL-α-tocopheryl phosphate diester potassium salt	100 mg
. Lactose	80 mg
. Corn starch	17 mg
. Magnesium stearate	3 mg
	Total 200 mg (a tablet)

Claims

1. Use of an effective amount of a compound of the formula:



wherein R_1, R_2 and R_3 are independently the methyl group or a hydrogen atom, or a salt thereof in admixture with a pharmaceutically acceptable carrier therefor for the manufacture of a pharmaceutical composition for the prophylaxis or treatment of ischemic disorder in mammalian organs.

2. Use according to claim 1, wherein at least one of R_1 and R_2 is the methyl group and R_3 is the methyl group in the compound.

3. Use according to claim 1, wherein the compound is a member selected from L-ascorbic acid, DL-α-tocopheryl phosphate diester, its potassium salt, its sodium salt or its calcium salt; L-ascorbic acid, DL-β-tocopheryl phosphate diester or its sodium salt; L-ascorbic acid, D-γ-tocopheryl phosphate diester or its sodium salt; L-ascorbic acid, D-δ-tocopheryl phosphate diester or its potassium salt; and L-ascorbic acid, tocoryl phosphate diester ora its sodium salt.

4. Use according to claim 3, wherein the compound is L-ascorbic acid, DL-α-tocopheryl phosphate diester sodium salt.

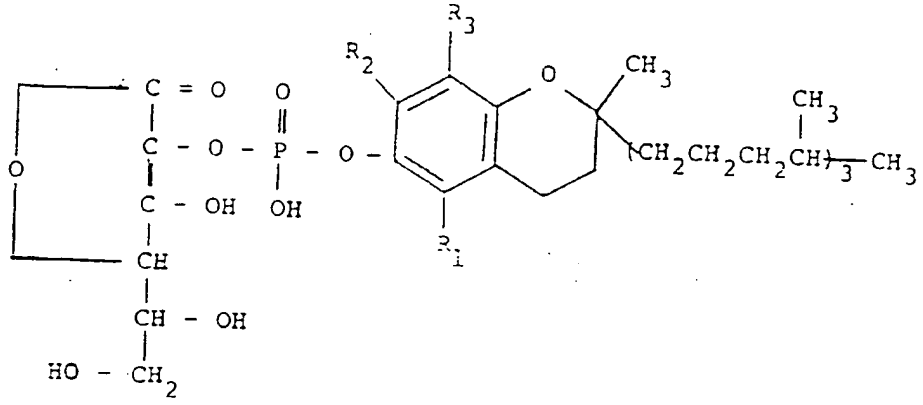
5. Use according to claim 1, which contains the compound in an amount of 5 to 1,000 mg.

6. Use according to claim 1, which is in the form of a tablet.

7. Use according to claim 1, wherein the ischemic disorder in mammalian organs is ischemic heart disease, ischemic disorder of cerebral tissue or ischemic renal disorder.

Patentansprüche

1. Verwendung einer wirksamen Menge einer Verbindung der Formel



worin R_1, R_2 und R_3 unabhängig voneinander die Methylgruppe oder ein Wasserstoffatom darstellen, oder eines Salzes derselben in Mischung mit einem pharmazeutisch verträglichen Trägermittel dafür zur Herstellung einer pharmazeutischen Zusammensetzung zur Prophylaxe oder Behandlung von ischämischen Störungen in den Organen von Säugetieren.

2. Verwendung nach Anspruch 1, worin wenigstens eines von R_1 oder R_2 die Methylgruppe und R_3 die Methylgruppe in der Verbindung ist.

3. Verwendung nach Anspruch 1, worin die Verbindung ein Mitglied ist, das aus L-Ascorbinsäure-DL-α-Tocopherylphosphatdiester, seinem Kaliumsalz, seinem Natriumsalz oder seinem Kalziumsalz; L-Ascorbinsäure-DL-β-tocopherylphosphatdiester oder seinem Natriumsalz; L-Ascorbinsäure-D-γ-tocopherylphosphatdiester oder seinem Natriumsalz; L-Ascorbinsäure-D-δ-tocopherylphosphatdiester oder seinem Kaliumsalz; und L-Ascorbinsäuretocopherylphosphatdiester oder seinem Natriumsalz ausgewählt ist.

4. Verwendung nach Anspruch 3, worin die Verbindung L-Ascorbinsäure-DL-α-tocopherylphosphatdiesternatriumsalz ist.

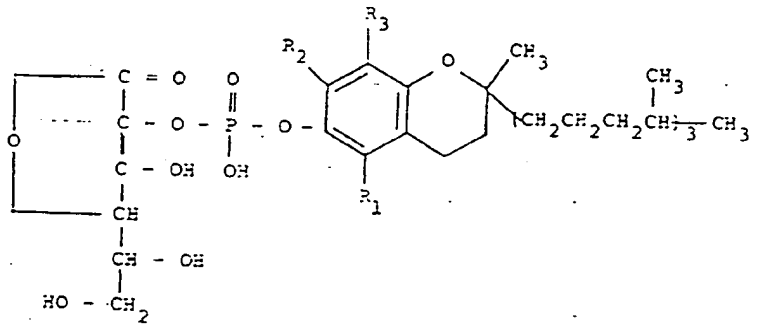
5. Verwendung nach Anspruch 1, worin die Verbindung in einer Menge von 5 bis 1.000 mg enthalten ist.

6. Verwendung nach Anspruch 1, die in Form einer Tablette erfolgt.

7. Verwendung nach Anspruch 1, worin die ischämische Störung in den Organen von Säugetieren eine ischämische Herzkrankheit, eine ischämische Störung des Gehirngewebes oder eine ischämische Nierenstörung ist.

Revendications

1. Emploi d'une quantité efficace d'un composé de formule



dans laquelle R_1, R_2 et R_3 représentent indépendamment chacun un groupe méthyle ou un atome d'hydrogène,
ou d'un sel d'un tel composé, mélangé avec un véhicule acceptable en pharmacie, pour la fabrication d'une composition pharmaceutique destinée à la prophylaxie ou au traitement de troubles ischémiques d'organes de mammifères.

2. Emploi conforme à la revendication 1, dans lequel, dans le composé, au moins l'un de R_1 et R_2 représente un groupe méthyle et R_3 représente un groupe méthyle.

3. Emploi conforme à la revendication 1, dans lequel le composé est choisi parmi le diester phosphate d'acide L-ascorbique et de DL-α-tocophérol, son sel de potassium, son sel de sodium et son sel de calcium, le diester phosphate d'acide L-ascorbique et de DL-β-tocophérol et son sel de sodium, le diester phosphate d'acide L-ascorbique et de D-γ-tocophérol et son sel de sodium, le diester phosphate d'acide L-ascorbique et de D-δ-tocophérol et son sel de potassium, et le diester phosphate d'acide L-ascorbique et de tocorol et son sel de sodium.

4. Emploi conforme à la revendication 3, dans lequel le composé est le sel de sodium du diester phosphate d'acide L-ascorbique et de DL-α-tocophérol.

5. Emploi conforme à la revendication 1, pour une composition contenant de 5 à 1000 mg dudit composé.

6. Emploi conforme à la revendication 1, pour une composition sous forme de comprimés.

7. Emploi conforme à la revendication 1, les troubles ischémiques d'organes de mammifères étant la maladie cardiaque ischémique, les troubles ischémiques des tissus cérébraux ou les troubles rénaux ischémiques.